Infrared Spectra and Protein Conformations in Aqueous Solutions

I. THE AMIDE I BAND IN H_2O AND D_2O SOLUTIONS

H. SUSI, SERGE N. TIMASHEFF,* AND LINDA STEVENS

From the U. S. Department of Agriculture, Eastern Utilization Research and Development Division, Philadelphia, Pennsylvania 19118

SUMMARY

Infrared absorption spectra of poly-L-lysine, poly-L-glutamic acid, β-lactoglobulin, myoglobin, and α_s-casein in the region of absorption of the amide I band have been observed in H_2O solution, D_2O solution, and in the solid state. The results indicate that characteristic frequencies exhibited by specific conformations of the investigated synthetic polypeptides are not transferable to corresponding conformations of globular proteins. The frequencies obtained for different conformations of globular proteins in H_2O and D_2O solution are internally consistent, in general agreement with corresponding values of fibrous proteins and with the limited data available in the literature concerning deuterated proteins in D_2O solution. Dissolution in aqueous environment by itself does not noticeably alter the amide I frequencies. A tentative set of characteristic frequencies and interaction constants is obtained for the amide I′ modes of *N*-deuterated proteins. These modes are easily observed in D_2O solution and show sufficient variations in frequency to permit a distinction between the α-helical, the antiparallel-chain pleated sheet, and the solvated random configurations of globular proteins.

Infrared absorption spectroscopy has been widely used in studies concerned with the secondary structure of fibrous proteins and synthetic polypeptides in the solid state (1–7). Miyazawa (5) has analyzed the amide I and amide II bands (absorbing between ~1610 and 1690, and ~1520 and 1550 cm^{-1}, respectively) in terms of a weakly coupled oscillator model, and proposed a procedure for interpreting the fine structure and polarizaation of these bands in terms of different polypeptide conformations. Miyazawa and Blout (6) applied the results of the theory to observed spectra of polypeptides and fibrous proteins, and proposed numerical values of pertinent physical constants for the α-helical conformation, the antiparallel chain-pleated sheet conformation (8) and the parallel-chain pleated sheet conformation (8). The procedure has been modified by Krimm (7), and extended to cover the polar chain conformation (9) and the polyglycine II type triple helix (10). It has been possible to analyze the conformation of a considerable number of fibrous proteins on the basis of these calculations (7).

The present communication examines the possibility of applying an analogous approach to polypeptides and globular proteins in aqueous environment. Experimental difficulties frequently make it necessary to carry out measurements in D_2O solution, since H_2O exhibits strong absorption in the spectral region of interest (11). In order to interpret the data obtained in D_2O solution, and to compare the results with previous work carried out on solid samples, it is necessary to evaluate the effect of dissolution as well as the effect of hydrogen-deuterium exchange in the peptide groups. Because no polarization measurements can be carried out in solution, interpretation must be based on frequency criteria alone.

Previous work reported on certain synthetic polypeptides (3, 12, 13) and some proteins (14, 15) has shown that amide I′ frequencies can provide information concerning peptide conformation in D_2O solution. In order to obtain additional insight, and to clarify some apparent inconsistencies, we have examined several synthetic polypeptides and proteins of known conformation in D_2O solution, H_2O solution, and in the solid state, and analyzed the results in the light of the approach originally proposed by Miyazawa (5). Particular attention was given to possible differences between spectral (and structural) characteristics of some synthetic polypeptides on one hand and globular proteins on the other. In contrast to preliminary studies (14), investigations in D_2O solution were carried out after completion of hydrogen-deuterium exchange in the peptide groups, in order to obtain uniform data and to evaluate the feasibility of obtaining characteristic frequencies and intergroup interaction constants (5–7) for *N*-deuterated proteins.

Subsequently a number of globular proteins were studied in D_2O solution to obtain information on their structural character-

* Present address, Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154.

istics in aqueous medium, and the denaturation of β-lactoglobulin in aqueous environment was investigated by infrared absorption. These results are reported in the companion paper (16).

EXPERIMENTAL PROCEDURE

All infrared measurements were carried out with a Beckman[1] IR-7 spectrophotometer, calibrated with the help of atmospheric water vapor bands. Instrumental accuracy and precision was better than ± 0.5 cm^{-1}. The uncertainty in reported frequency values is somewhat larger, about ± 1 to 2 cm^{-1} in D_2O solution, because of the broadness of some observed bands and of uncertainties caused by overlapping absorption bands. Matched sealed cells of conventional design, with 0.1-mm path length and CaF_2 windows, were used for measurements in D_2O solution. The reference cell was filled with pure D_2O. It is extremely difficult to eliminate traces of HOD in D_2O solutions of proteins and polypeptides because the slightest contact with atmosphere will cause exchange in the solvent, in addition to the exchange caused by deuteration of the peptide groups. (On the other hand, the amount of H_2O remains very small, because of the favorable equilibrium: $H_2O + D_2O \rightleftarrows 2HOD$.) The bending mode of HOD (17) absorbs very close to the amide II′ band (18) of *N*-deuterated polypeptides and proteins. Because of this interference, precise measurements of amide II′ frequencies are extremely difficult in D_2O solution and attention is focused in this communication on amide I′ frequencies. (The characteristic frequencies of *N*-deuterated secondary amides, polypeptides, and proteins are conventionally labeled amide I′, amide II′ and so on (18).)

Spectra in H_2O solution were obtained with sealed cel's of variable path length equipped with CaF_2 windows (model VT-01, Research and Industrial Instruments Company, London, England).[1] H_2O absorbs very strongly in the 1500 to 1800 cm^{-1} region in which amide I and amide II bands are observed (11). As an example of the problems encountered, spectra of H_2O and a 50 g per liter solution of myoglobin in H_2O obtained in a 0.01-mm cell are shown in Fig. 1. As a result, measurements of amide I and amide II frequencies in H_2O solution are possible only by extremely careful differential procedures; these are not easily adopted for routine investigations. Absorption by the solvent was cancelled by repeatedly adjusting the path length of the reference cell and measuring the differential absorption at various wave length settings until a spectrum was obtained which showed only characteristic polypeptide bands. To prevent aggregation of the solute, it was necessary to use dilute solutions, while the intense H_2O absorption precluded the use of cell thicknesses larger than 0.01 mm. An ordinate scale expansion device, which forms an integral part of the employed spectrophotometer, was therefore used. Although data obtained in this manner are not as precise as corresponding data obtained in D_2O solution, repeated experiments led to reproducible and internally consistent results. The tedious and time-consuming experiments were carried out in order to obtain information concerning the effect of dissolution of proteins in water without simultaneously introducing additional variables associated with deuteration.

Spectra of deposited films and of mineral oil mulls were obtained in a conventional manner.

[1] Mention of the above product does not imply endorsement by the United States Department of Agriculture over others not mentioned.

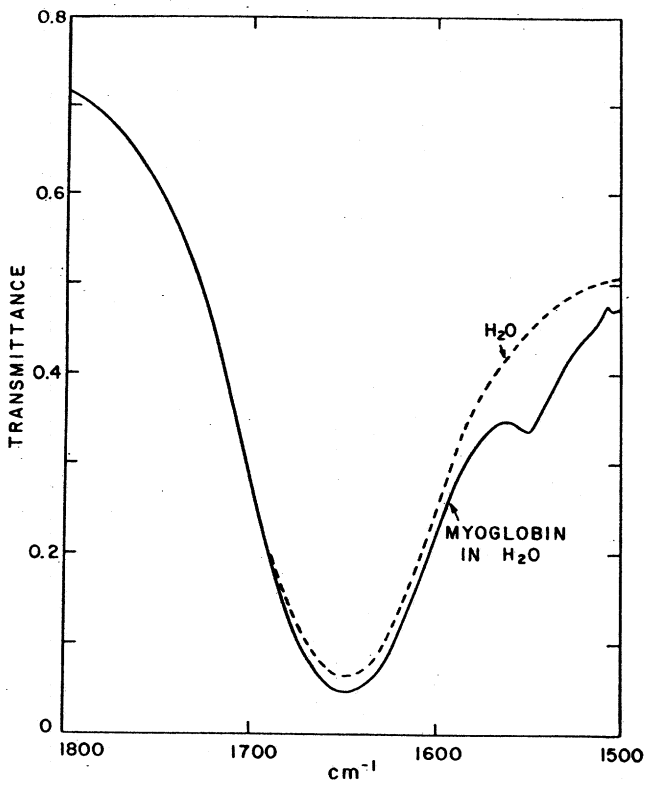

FIG. 1. Infrared spectra in the 1,650 cm^{-1} region of H_2O and a 50 g per liter solution of myoglobin measured in a cell with a path length of 0.01 mm.

The experimental conditions which were used to obtain poly-L-lysine in the α-helical, the antiparallel-chain pleated sheet, and the unordered conformation in dilute H_2O solution have been described (19). Precisely analogous procedures were used to obtain the corresponding conformations in D_2O solution. The preparation of D_2O solutions of α-helical myoglobin, native and denatured β-lactoglobulin, and of α_s-casein has also been described (14). In a previous communication (14), spectra of native myoglobin and β-lactoglobulin were reported which were obtained in D_2O solution before considerable hydrogen-deuterium exchange had taken place in the peptide groups. To obtain a uniform set of data suitable for evaluating pertinent constants of deuterated proteins, and to avoid complications caused by possible partial exchange, the spectra of all samples in D_2O solution were obtained after virtually complete exchange had taken place. Myoglobin and β-lactoglobulin were dissolved in D_2O at pD[2] values of 7.0 and 7.7, respectively, at which exchange is known to proceed essentially to completion (20–23) and kept at the same pD for 48 hours at 25° prior to the spectroscopic experiment; α_s-casein was dissolved at pD 10.9 and also kept for 48 hours, even though, for that protein, the amide II band disappeared very rapidly. Hydrogen-deuterium exchange in the peptide groups was followed by observing the absorbance of the amide II band, as described by Blout, de Lozé, and Asadourian (23) and found to be essentially complete after 48 hours. In the case of β-lactoglobulin, an experiment was carried out, furthermore, in

[2] The pD values are those actually read on a pH meter in D_2O solution and might involve error of about 0.4 unit. They are selected so that a change by that amount would not lead to conformational changes (14).

which the protein, after 48 hours of exposure to pD 7.7 at 25°, was brought down to pD 2.1. The spectrum of the resulting solution was compared with that of a freshly prepared solution with a pD of 2.1 in which only partial exchange could have taken place (22–24), with the results shown in Fig. 4, *Curves a* and *b*; these will be discussed in detail later. H_2O solutions of β-lactoglobulin, myoglobin, and α_s-casein were prepared by the same procedures which were used to prepare the solutions in deuterium oxide. D_2O solutions of poly-L-glutamic acid were prepared by dissolving a weighed out amount of the polymer in D_2O and adjusting the pD value to 4.3 for the α-helical and 7.0 for the unordered conformation.

RESULTS

Some Pertinent Relationships—Interpretation of the experimental results was based on the approach described by Miyazawa (5) and by Miyazawa and Blout (6), as extended and modified by Krimm (7), and critically discussed by Bradbury and Elliott (25). To permit a coherent presentation and discussion of the obtained data, some pertinent relationships and approximations used during the course of this work are briefly summarized. Considerations are limited to amide I and amide I′ bands because no precise data could be obtained on amide II′ bands in D_2O solution (*cf.* "Experimental Procedure").

In ordered polypeptide conformations, vibrational coupling between neighboring peptide groups causes the amide I (and amide I′) band to split into a number of branches (5–7). The frequency of the strongest branch of the α-helical conformation is given by (7)

$$\nu(0)_\alpha = \nu_0 + D_1 + D_2 + D'_1 \qquad (1)$$

The antiparallel-chain pleated sheet conformation gives rise to two easily recognized amide I branches (5)

$$\nu(0,\pi)_A = \nu_0 + D_1 - D'_1 \quad \text{(weak)} \qquad (2)$$

$$\nu(\pi,0)_A = \nu_0 - D_1 + D'_1 \quad \text{(strong)} \qquad (3)$$

ν_0 is the unperturbed frequency of a single oscillator; D_1 is the intrachain interaction constant between nearest neighbors, D_2 the corresponding constant between second neighbors, and D'_1 is the interchain interaction term between nearest neighbors across hydrogen bonds.

It is commonly assumed (6, 7) that ν_0 is transferable from one conformation and from one protein to another for solid samples. Krimm makes the further assumption that D_1 and D'_1 are similarly transferable (with proper signs) (7). D_2 in Equation 1 has a very low numerical value, 2 cm^{-1}, for solid samples (7). If this value is carried over to H_2O and D_2O solutions, it becomes possible to calculate ν_0, D_1, and D'_1 by Equations 1 to 3 from three easily assigned bands arising from known configurations. These values, in turn, can be used to obtain frequency values for weak bands and for other conformations (7). This procedure is admittedly approximate, because the D constants involve potential and kinetic energy terms (5), the latter being sensitive to isotopic substitution. However, simple consideration shows that even a ± 100% error in D_2 will lead to only 1 cm^{-1} errors in calculated values of D_1 and D'_1, and to frequency errors not exceeding 2 cm^{-1}. The common procedure of obtaining the constants from very simple models (6, 7) results in uncertainties which are of the same magnitude or larger (7). Values for ν_0 and for the quantity $(D_1 - D'_1)$ are obtained from Equations 2 and 3 without any approximations except the ones inherent in the basic theory (5). It should be noted that for ordered structures in solution ν_0 is not necessarily close to the observed frequency of unordered conformations because of possible solvent-solute interactions in the latter (18).

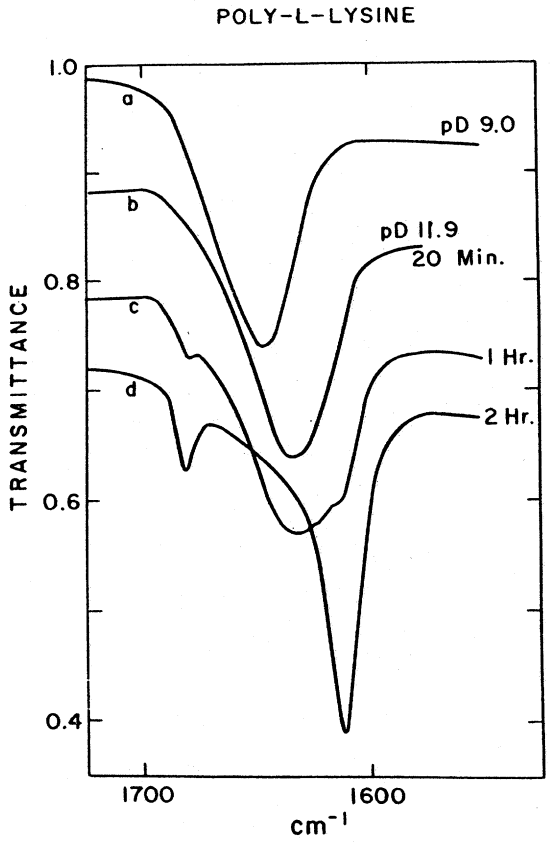

FIG. 2. The amide I′ band of poly-L-lysine in D_2O solution. Path length, 0.1 mm; concentration, 0.4% by weight. Pure D_2O as reference. Consecutive spectra displaced by 0.1 scale unit (*upper curve* is on scale). *a*, random conformation; *b*, α-helix; *c*, transition between α-helix and antiparallel-chain pleated sheet; *d*, antiparallel-chain pleated sheet.

Synthetic Polypeptides—Fig. 2 shows the amide I′ band of poly-L-lysine in the random conformation, the α-helical conformation, in a partially converted state between the α-helical and the antiparallel-chain pleated sheet conformation, and in the antiparallel-chain pleated sheet conformation as observed in dilute D_2O solution. The conformations of the uniform samples were checked by circular dichroism (19). It is evident that, for this simple model peptide, the three conformations are easily distinguishable by amide I′ frequencies, and that approximate values can be obtained for the composition of antiparallel-chain pleated sheet, α-helix mixtures. The antiparallel-chain pleated sheet conformation was also studied in H_2O solution (2% by weight, pH 11) and in solid (nondeuterated) films. The obtained $\nu(0, \pi)$ and $\nu(\pi, 0)$ frequencies, which are easily observed and assigned in H_2O solution and in solid film, are given in Table I, together with calculated values of ν_0 and the quantity $(D_1 - D'_1)$. Recent data on polyglycine films (26) are included for comparison.

Inspection of the data given in Table I leads to some noteworthy observations. First, the frequencies observed in the

TABLE I

Amide I and amide I′ frequencies of poly-L-lysine in antiparallel-chain pleated sheet conformation

Data of polyglycine I (26) included for comparison. Nomenclature is as in Reference 7.

Sample and state	$\nu(0, \pi)_A$	$\nu(\pi, 0)_A$	ν_0 (calculated)	$D_1 - D'_1$ (calculated)
Poly-L-lysine				
D_2O solution	1680	1611	1646	35
H_2O solution	1690	1616	1653	37
Film	1693	1625	1659	34
Polyglycine I				
Film (26)	1685	1636	1661	25
N-Deuterated polyglycine I				
Film (26)	1680	1629	1655	26

solid state and in H_2O solution are significantly different. The difference in ν_0 is 6 cm^{-1}, implying that the unperturbed force constant $F_{k,k}$ (5) and the strength of hydrogen bonding are different in the two states. (The frequency of C=O stretching modes is generally lowered by increasing the strength of hydrogen bonding (27).) In the solid state an intermolecular antiparallel-chain pleated sheet structure can be formed; in dilute solution the antiparallel-chain pleated sheet structure could be a result of folding of the same polymer molecule. The detailed structure of these related conformations appears to be slightly different. It is interesting to note that slightly different frequencies for antiparallel-chain pleated sheet conformations of the same polymer have also been observed in solid films of sodium poly-L-glutamate at different humidities (28). This could be caused by interactions of —C=O···HN— with water (29). Evidently caution must be exercised in transferring ν_0 values from one environment to another.

Second, the value of $(D_1 - D'_1)$ (equal to the sum of the absolute values, because the numerical value of D'_1 is negative (6, 7)) is essentially invariant for poly-L-lysine under all studied conditions, but different from the corresponding value for polyglycine I and for most fibrous proteins (7). Even though individual D_1 and D'_1 values cannot be obtained without further approximations (as discussed above), it is evident that the interaction constants can be substantially changed by the nature of side chains, as suspected by Krimm (7). They can evidently not be transferred from the antiparallel-chain pleated sheet structure of poly-L-lysine films to the antiparallel-chain pleated sheet structure of polyglycine films.

Third, the change of the unperturbed frequency, ν_0, upon *N*-deuteration is ~6 cm^{-1} for poly-L-lysine in solution as well as for polyglycine in the solid state. This shift does not appear to be sensitive to side chains and is retained for proteins, as discussed in the next section.

Table II lists frequencies for the α-helical and unordered conformations of poly-L-lysine and poly-L-glutamic acid under experimental conditions in which meaningful and reproducible values could be obtained. For the α-helical conformation in D_2O solution, the frequency of the strongest band, $\nu(0)_\alpha$, again depends on the nature of the side chains. It increases from poly-L-lysine to poly-L-glutamic acid and has an even higher value for the proteins which will be discussed subsequently. It appears that the observed frequencies of the investigated synthetic polypeptides in D_2O solution are not a reliable guide for corresponding values of globular proteins. The CONH groups of simple polypeptides are probably in closer contact with the solvent and in a different dielectric environment. Subtle differences between the backbone structure of corresponding conformations of simple polypeptides and proteins (resulting in slightly different force fields) can also not be ruled out. Since poly-L-lysine can be obtained in the antiparallel-chain pleated sheet as well as the α-helical conformation in dilute D_2O solution, it is possible to estimate individual values of D_1 and D'_1 for this system. Application of Equations 1, 2, and 3 yields $D_1 = +11$ cm^{-1}, $D'_1 = -24$ cm^{-1}, if D_2 is assumed to have a value of 2 cm^{-1}. The values are of interest for comparison with proteins under similar conditions.

Proteins—Fig. 3 shows the infrared spectra of native β-lactoglobulin, alkali-denatured β-lactoglobulin, α_s-casein, and myoglobin in H_2O solution.

The formidable experimental difficulties encountered in H_2O solution were already discussed under "Experimental Procedure." The results are not as precise as the ones obtained in D_2O solution or in the solid state. They do furnish, however, some badly needed information concerning the effects of an aqueous environment, without the additional complications caused by deuteration. The amide I and amide II frequencies of myoglobin are very close to the values which have been calculated and observed (7) for solid fibrous proteins in the α-helical conformation, and identical with corresponding values of crystalline myoglobin, as observed in mineral oil mulls.

The bands of native β-lactoglobulin in H_2O solution are also very similar to corresponding bands (14) observed in the solid state. The frequency of the strongest component of the amide I band, 1632 cm^{-1}, is identical with the value calculated for the nonpolar pleated sheet structures of fibrous proteins (7), and identical with the value obtained for β-lactoglobulin in the dry film state (14). Although measurements in H_2O solution involve uncertainties which are not present under more favorable experimental conditions (*cf.* "Experimental Procedure"), these observations strongly suggest that for ordered protein conformations dissolution in H_2O does not alter the characteristic frequencies. (They also lend support to the previous conclusion that, in the case of poly-L-lysine, two related but different antiparallel chain-pleated sheet conformations are observed in the solid state and in dilute solution, respectively.) The 1656 cm^{-1} amide I frequency of denatured β-lactoglobulin and of α_s-casein appears to be characteristic for an unordered solvent-penetrable conformation of proteins in H_2O solution. (There is no direct counterpart for this frequency in solid samples.)

TABLE II

Amide I and amide I′ frequencies of poly-L-lysine and poly-L-glutamic acid in α-helical and unordered conformations

Sample	$\nu(0)_\alpha$	ν (unordered)
Poly-L-lysine		
D_2O solution	1635	1645
Poly-L-glutamic acid		
D_2O solution	1640	1643
Film (nondeuterated)	1652	1657

Fig. 4 presents data observed in D_2O solution. *Curves a* and *b* show the amide I and amide I′ band of native β-lactoglobulin in D_2O solution, as observed before and after hydrogen-deuterium exchange in the peptide groups. (Note the disappearance of the amide II band in the 1550 cm^{-1} region of the deuterated sample.)

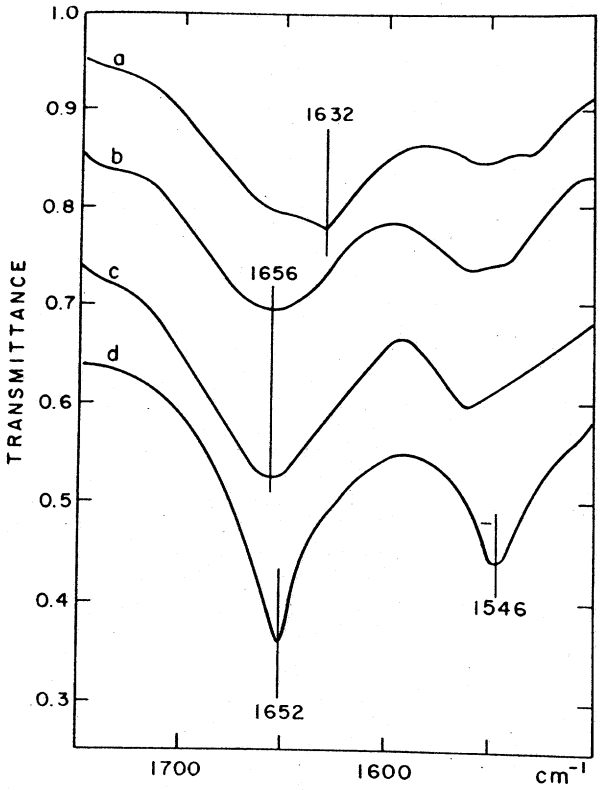

FIG. 3. Spectra of proteins in aqueous solution. Concentration, 3% by weight. Path length, about 0.01 mm. Pure H_2O as reference. ×2 ordinate scale expansion. Displacement of curves as in Fig. 2. *a*, native β-lactoglobulin, pH 6.5; *b*, denatured β-lactoglobulin, pH 12.3; *c*, α_s-casein, pH 10.6; *d*, myoglobin, pH 7.6.

For the nondeuterated protein, amide I bands associated with the antiparallel-chain pleated sheet β-structure are observed at 1632 cm^{-1} (medium to strong) and at 1690 cm^{-1} (weak). For the deuterated protein, corresponding amide I′ frequencies occur at 1632 cm^{-1} and 1675 cm^{-1} (very weak shoulder). The strong $\nu(\pi, 0)_A$ branches of antiparallel chain-pleated sheet bands are not influenced by deuteration, but the weak $\nu(0, \pi)_A$ branch shifts to a lower frequency. By applying Equations 2 and 3 one obtains ν_0 (amide I) = 1661 cm^{-1}; ν_0 (amide I′) = 1654 cm^{-1}. The shift is in excellent agreement with the corresponding shift

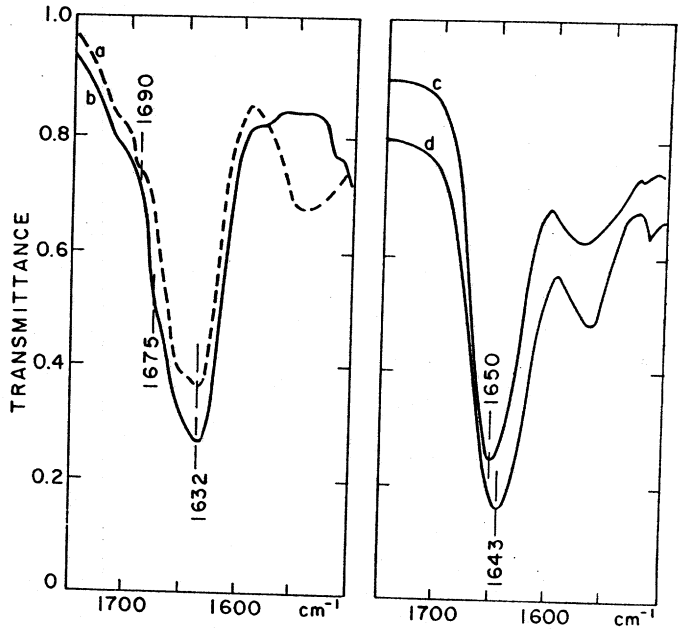

FIG. 4. Spectra of *N*-deuterated proteins in D_2O solution. Concentration, 2% by weight. Path length, 0.1 mm. Pure D_2O as reference. *a*, β-lactoglobulin, pD 2.1, before exchange; *b*, β-lactoglobulin, pD 2.4, after exchange; *c*, myoglobin, pD 7.0; *d*, α_s-casein, pD 10.9 (displaced by 0.1 scale unit).

TABLE III

Observed amide I and amide I′ frequencies of proteins

Conformation	Mode	State			Sample
		D_2O solution	H_2O solution	Solid	
Antiparallel-chain pleated sheet	$\nu(\pi, 0)_A$	1632	1632	1632	β-Lactoglobulin
				1630–1634[a]	Fibrous proteins
	$\nu(0, \pi)_A$	1675	1690[b]	1690	β-Lactoglobulin
				1695–1697[a]	Fibrous proteins
α-Helix	$\nu(0)\alpha$	1649[c]			β-Lactoglobulin
		1650	1652	1652	Myoglobin
				1650–1653[a]	Fibrous proteins
Unordered	$\nu(u)$[d]	1643	1656		β-Lactoglobulin
		1643	1656		α_s-Casein
				1660–1664[a]	Fibrous proteins

[a] From Reference 7, for comparison.
[b] Unexchanged protein in D_2O solution.
[c] In D_2O-CH_3OD mixed solvent.
[d] Observed value for unordered form; not necessarily equal to ν_0.

obtained for poly-L-lysine and polyglycine (see above), suggesting that the very weak shoulder at 1675 cm^{-1} has been correctly identified. (It can only be observed by very slow scanning under optimum instrumental conditions.) The difference between the $\nu(0, \pi)_A$ intensities of the nondeuterated and deuterated samples is not surprising, because the nature of the amide I and amide I′ vibrations is slightly different (18); the mode would not be observed at all for pure C=O stretching vibrations of parallel groups.

Curve c in Fig. 4 shows the amide I′ band of myoglobin in D_2O solution (the weak band at ~1575 cm^{-1} is caused by ionized side chain carboxyl groups). Again the frequency of the strongest amide I component is only very slightly influenced by *N*-deuteration, as seen by comparison with Fig. 3. *Curve d* gives the amide I′ band of α_s-casein. The corresponding band of denatured β-lactoglobulin is practically identical with the α_s-casein band. β-Lactoglobulin was also investigated in a mixed solvent of 90% CH_3OD and 10% D_2O, which results in a predominately α-helical conformation for this protein (30). Solid state spectra were obtained of native nondeuterated β-lactoglobulin and myoglobin. The values of all observed amide I and amide I′ frequencies are collected in Table III; some literature values (7, 15) are included for comparison.

DISCUSSION

Comparison of Table III with data presented in Tables I and II reveals that the amide I′ frequencies of the investigated proteins in D_2O solution are significantly different from corresponding values of the synthetic polypeptides, poly-L-lysine and poly-L-glutamic acid. Somewhat analogous phenomena have also been observed by previous workers on nondeuterated solid films. For instance, oriented films of sodium poly-L-glutamate lead to amide I frequencies for α-helical and antiparallel chain-pleated sheet conformations (28) which are quite different from values obtained for fibrous proteins (6, 7). The frequencies of the fibrous proteins themselves, on the other hand, exhibit relatively small variations (7). The frequency range is given in Table III. (Although the relative amounts of various side chains differ from protein to protein, the variations are evidently less drastic than between polypeptides composed of uniform amino acid residues.)

The values obtained in this study for globular proteins in the solid state and in H_2O solution agree well with corresponding values of fibrous proteins. This implies that characteristic amide I frequencies for different conformations are relatively constant for proteins in general and that dissolution in aqueous environment does not have an appreciable effect on the frequencies of ordered protein conformations. The number of globular proteins of known structure, which could be used to check the transferability of the reported frequencies, is unfortunately rather limited. Hamaguchi (15) and Fukushima, as cited by Hamaguchi (15), have obtained amide I′ values for lysozyme in D_2O solution which are in very good agreement with our data ($\nu(0)_\alpha$ = 1650 cm^{-1}, $\nu(\pi, 0)_A$ = 1630 − 1632 cm^{-1}), although the amide I′ band of the latter protein is not easily resolved (15). If the proteins examined in this study, and lysozyme, are representative of globular proteins in D_2O solution, characteristic frequencies and interaction constants can be calculated by Equations 1, 2, and 3, as discussed.

At present, the results of such calculations must be regarded as tentative because of the limited amount of available experimental data. The numerical results are, nevertheless, of interest because they provide insight concerning the apparent stability of some strong amide I branches upon deuteration. Assigning $\nu(0)_\alpha$ = 1650, $\nu(\pi, 0)_A$ = 1632 and $\nu(0, \pi)_A$ = 1675 cm^{-1} (Table III), we obtain for the amide I′ mode in D_2O solution

$$\nu_0(\text{amide I}') = 1654\ cm^{-1};\quad D_1 = 8;\quad D'_1 = -14$$

Also from the data in Table III we obtain for H_2O solution and the solid state

$$\nu_0(\text{amide I}) = 1661\ cm^{-1};\quad D_1 = 9;\quad D'_1 = -20$$

(The latter values are in good agreement with Krimm's values for fibrous proteins (7): ν_0 = 1658 cm^{-1}; D_1 = 8; D'_1 = −18.)

The apparent constancy of the strong $\nu(0)_\alpha$ and $\nu(\pi, 0)_A$ modes upon deuteration of the peptide groups thus appears to be a consequence of equal but opposite shifts of the numerical values of ν_0 and D'_1. The shift of ν_0 reflects decoupling of the amide I and amide II modes in a single polypeptide group upon deuteration (18). The change in D'_1 reflects different coupling between groups linked across N—H···O=C in nondeuterated systems and across N—D···O=C in deuterated systems.

From the values of ν_0, D_1, and D'_1 (assuming that D_2 = 2, as discussed), the following set of tentative frequencies is obtained for the amide I′ bands of different protein conformations in D_2O solution (*, experimental frequencies; *s*, strong; *w*, weak; *vw*, very weak)

Antiparallel-chain pleated sheet

$$\nu(0, \pi) = 1675^*\ (w),\quad \nu(\pi, 0) = 1632^*\ (s)$$

$$\nu(\pi, \pi) = 1660\ (vw)$$

Parallel-chain pleated sheet

$$\nu(0, 0) = 1648\ (w),\quad \nu(\pi, 0) = 1632\ (s)$$

Polar chain

$$\nu(0, 0) = 1648\ (s),\quad \nu(\pi, 0) = 1632\ (w)$$

α-Helix

$$\nu(0) = 1650^*\ (s),\quad \nu(2\pi/3.6) = 1644\ (w)$$

Solvated unordered form

$$\nu(u) = 1643^*(s)$$

For the polyglycine II triple helix structure no predictions are possible at present because D_2 has a high value for this conformation (7). Since dissolution in H_2O does not appear to influence characteristic frequencies of ordered protein conformations, and there is no reason to assume that D_2O behaves differently in this respect, the listed values should also hold for solid *N*-deuterated proteins.

The amide I and amide I′ frequencies of unordered proteins in H_2O and D_2O solution remain to be discussed. It has been suggested (18) that in certain synthetic polypeptides COND groups solvated by D_2O give rise to an amide I′ band around 1665 cm^{-1}. No such band of observable intensity could be detected in denatured β-lactoglobulin or the unordered protein α_s-casein. The ν_0 value obtained from β-lactoglobulin in the antiparallel chain-pleated sheet configuration (without any approximations not inherent in the basic theory (5)) is 1654 cm^{-1}. A similar value would be expected for a random nonsolvated form (6, 7). The observed value in D_2O solution is 1643 cm^{-1}. We are inclined to assign it to solvated *N*-deuterated peptide groups

in D_2O solution. The corresponding value in H_2O solution is 1656 cm^{-1}.

REFERENCES

1. Elliott, A., and Ambrose, E. J., *Nature*, **165,** 921 (1950).
2. Elliott, A., *Proc. Roy. Soc. (London), Ser. A.*, **221,** 104 (1954).
3. Doty, P., Imahori, K., and Klemperer, E., *Proc. Nat. Acad. Sci. U. S. A.*, **44,** 424 (1958).
4. Beer, M., Sutherland, G. B. B. M., Tanner, K. N., and Wood, D. L., *Proc. Roy. Soc. (London), Ser. A.*, **249,** 147 (1959).
5. Miyazawa, T., *J. Chem. Phys.*, **32,** 1647 (1960).
6. Miyazawa, T., and Blout, E. R., *J. Amer. Chem. Soc.*, **83,** 712 (1961).
7. Krimm, S., *J. Mol. Biol.*, **4,** 528 (1962).
8. Pauling, L., and Corey, R. B., *Proc. Nat. Acad. Sci. U. S. A.*, **37,** 729 (1951).
9. Pauling, L. and Corey, R. B., *Proc. Nat. Acad. Sci. U. S. A.*, **37,** 251 (1951).
10. Crick, F. H. C., and Rich, A., *Nature*, **176,** 780 (1955).
11. Blout, E. R., *Ann. N.Y. Acad. Sci.*, **69,** 84 (1957).
12. Blout, E. R., and Idelson, M., *J. Amer. Chem. Soc.*, **80,** 4909 (1958).
13. Doty, P., Wada, A., Yang, J. T., and Blout, E. R., *J. Pol. Sci.*, **23,** 851 (1957).
14. Timasheff, S. N., and Susi, H., *J. Biol. Chem.*, **241,** 249 (1966).
15. Hamaguchi, K., *J. Biochem. (Tokyo)*, **56,** 441 (1964).
16. Timasheff, S. N. Susi, H., and Stevens, L., *J. Biol. Chem.*, **242,** 5467 (1967).
17. Swenson, C. A., *Spectrochim. Acta*, **21,** 987 (1965).
18. Miyazawa, T. in M. A. Stahmann (Editor), *Polyamino acids, polypeptides and proteins*, University of Wisconsin Press, Madison, Wisconsin, 1962, p. 201.
19. Townend, R., Kumosinski, T. F., Timasheff, S. N., Fasman, G. D. and Davidson, B., *Biochem. Biophys. Res. Commun.*, **23,** 163 (1966).
20. Benson, E. S., *Compt. Rend. Lab. Carlsberg, Ser. Chim.*, **31,** 235 (1959).
21. Beychok, S., De Lozé, C., and Blout, E. R., *J. Mol. Biol.*, **4,** 421 (1962).
22. Linderstrøm-Lang, K., *Soc. Biol. Chem. India, Souvenir*, 191 (1955).
23. Blout, E. R., De Lozé, C., and Asadourian, A., *J. Amer. Chem. Soc.*, **83,** 1895 (1961).
24. Klotz, I. M., and Frank, B. H., *J. Amer. Chem. Soc.*, **87,** 2721 (1965).
25. Bradbury, E. M., and Elliott, A., *Polymer*, **4,** 47 (1963).
26. Suzuki, S., Iwashita, Y., Shimanouchi, T., and Tsuboi, T., *Biopolymers*, **4,** 337 (1966).
27. Pimentel, G. C. and McClellan, A. L., *The hydrogen bond*, W. H. Freeman and Company, San Francisco, 1960.
28. Lenormant, H., Baudras, A., and Blout, E. R., *J. Amer. Chem. Soc.*, **80,** 6191 (1958).
29. Bendit, E. G., *Biopolymers*, **4,** 539, 561 (1966).
30. Timasheff, S. N., Townend, R., and Mescanti, L., *J. Biol. Chem.*, **241,** 1863 (1966).